Prediction and prevention of inbreeding depression by genetic markers - In the case of experimental fish guppy Poecilia reticulata

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学 位 の 種 類	博士（農学）
学 位 記 番 号	農博第 1119 号
学 位 授 与 年 月 日	平成 27 年 3 月 25 日
学 位 授 与 の 要 件	学位規則第 4 条第 1 項
研 究 科 ， 専 攻	東北大学大学院農学研究科（博士課程）生物産業創成科学専攻
論 文 題 目	Prediction and prevention of inbreeding depression by genetic markers - In the case of experimental fish guppy Poecilia reticulata（遺伝マーカーを用いた近交弱勢の予測と予防に関する研究　－ グッピーを用いたモデル実験 －）
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Prediction and prevention of inbreeding depression by genetic markers - In the case of experimental fish guppy *Poecilia reticulata*

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ABSTARCT

CHAPTER 1 - INTRODUCTION

The guppy (*Poecilia reticulata*) is a livebearer fish and living in freshwater topical weather. It has been used as model organism for more than a century. Several guppy traits have been cultured in our laboratory using close culture system. These strains were used for experiments since variation such as body colorization; sizes and growth rate exist between strains. Study on the effect of heterozygosity and fitness correlation still new to this species. Two strains consist of NA1 and AY strains were used in this experiment. General effect of inbreeding also known as inbreeding depression are common now days. However the effect of inbreeding on population and traits may varies. In this study, we used guppy as a model organism and genetic markers to analyzed, predict and possibly prevent the severe effect of inbreeding. In this presentation, seven chapters will be presented pertaining to the effect of full sibling mating and guppy fitness monitored by genetic information.

CHAPTER 2 – Observed depression in the process of inbred guppy

In this chapter, full-sibling mating were used to produced inbred guppy. During the inbred process, we documented the survival, growth, reproduction and mortality. AY and NA1 strain were used for this experiment. Effect of inbreeding to growth, reproduction and mortality were monitored during this experiment. Fitness related traits such as survival rate showed the severe effect of inbreeding depression. Mortality increased as the generation on inbred guppy increased (Table 1.1).

CHAPTER 3 – Thermal tolerance in inbred guppy

Thermal tolerances were examined in every generation at 37^{o}C and the survival times were recorded to evaluate the fitness of inbred guppy. Decreased in thermal tolerance were observed in NA1 and AY, which probably caused by inbreeding depression. The result suggested that producing high homogenous fish might have surfaced the unique homozygous recessive traits in each strain. High variation was observed in AY strains compared to NA1 strain. The NA1 strain seems to strive in control condition, however during the experiment the NA1 strain tolerance to heated condition are significantly lower than the AY strains (Figure 1.1 and figure 1.2)

CHAPTER 4 – Genetic changes during inbreeding process

Producing specific strains either for experimental or ornamental purposes may require the process of inbreeding, which involves mating with closely related individual. Usually this method will lead to inbreeding depression. To assess inbreeding, nine microsatellite markers were used. Observed and expected heterozygosity were calculated using microsatellite markers and fixation index were analyzed in every generations. Decreased in fixation index were observed as inbred generation progress. Dead samples were collected in the third and forth generations. By adding dead samples to live sample data, we detected decreased in observed heterozygosity proving that dead sample may increase the accuracy of calculating inbreeding level (Table 1.2 and 1.3). The standardize heterozygosity (SH) and internal relatedness (IR) were measured to examine the correlation between multilocus heterozygosity and coefficient if inbreeding. Significant correlation was observed indicating the use of microsatellite markers could possibly determine the inbreeding level in AY stain (Figure 1.3 and 1.4) and NA1 strains (Figure 1.5 and 1.6).

CHAPTER 5 – Correlation between multi locus heterozygosity and thermal tolerance

Heterozygosity had often been correlated with fitness relatively as the assumption that individuals with high heterozygosity are negatively correlated with inbreeding coefficient. In this study, we hypothesis that as the inbreeding increase the heat tolerance will decrease and probably producing lower heterozygosity progeny. AY and NA1 showed different tendency between thermal tolerance and individual heterozygosity. Correlation between SH and IR with thermal tolerance were done to determine the effect of inbreeding towards multilocus heterozygosity and fitness trait. NA1 showed significant correlation between SH and IR with thermal tolerance. This indicated only highly inbred strain generate significant correlation (Figure 1.7 and 1.8)

CHAPTER 6 – Relatedness

Parental relatedness and various traits of their offspring were compared. Only the AY strain illustrated significant correlation with progeny indicating relatedness may not be suitable to be used in inbred strain. This study illustrates the importance of relatedness in producing progeny. However, the inbreeding effect could possibly hinder the efficiency of relatedness calculation.

CHAPTER 7 – Discussion

Through the process on inbreeding, many potential parents were not able to reproduced increasing bottleneck effect. Fitness related traits were severely affected by inbreeding depression. Thermal tolerance and survival rate are traits that are affected during the inbreeding process. Fixation rate were recorded during the experiment. As the inbred generation progress, fixation index remain stable throughout the experiment. When dead inbred sample were added to the fixation index calculation, increased of fixation index were observed. Samples from deceased individual are important to estimate the true effect of inbreeding. Significant correlation between heat tolerance and

SH and IR were observed in this experiment. The SH and IR are an essential tools to determine the inbreeding depression. Fixation index can be used to estimate the overall inbred level by including deceased individual. This experiment illustrated the importance of genetic markers in avoiding and predicting inbreeding depression.

Table 1.1 - Inbred seed production separated by strains and generations. Parental generation (P) started with five pairs of male and female. The first generation (F_1) were cultured until it reached maturity and reproduced for several generations. In this experiment, up to fourth generation of full sibling inbred strain were produced. The number of survival individual decreased as the inbred generations increased. P: Parental generation, F_n: Inbred n generation, Family; no. of family, Invd; individual, AY; AY guppy strain, NA1; NA1 guppy strain, Surv%; percentage on survival individual.

AY				NA1			
Generation	Family	Invdl	Surv (%)±(SD)	Generation	Family	Invdl	Surv (%)±(SD)
P	5	10		P	5	10	
F_1	26	112	89.62 (22.71)^{a}	F_1	59	238	91.84 (15.21)^{a}
F_2	62	245	79.87 (28.91)^{ab}	F_2	56	230	79.01 (23.43)^{b}
F_3	29	148	79.87 (25.94)^{ab}	F_3	48	181	75.02 (22.25)^{bc}
F_4	35	112	74.71 (30.19)^{c}	F_4	58	243	73.79 (27.82)^{bc}

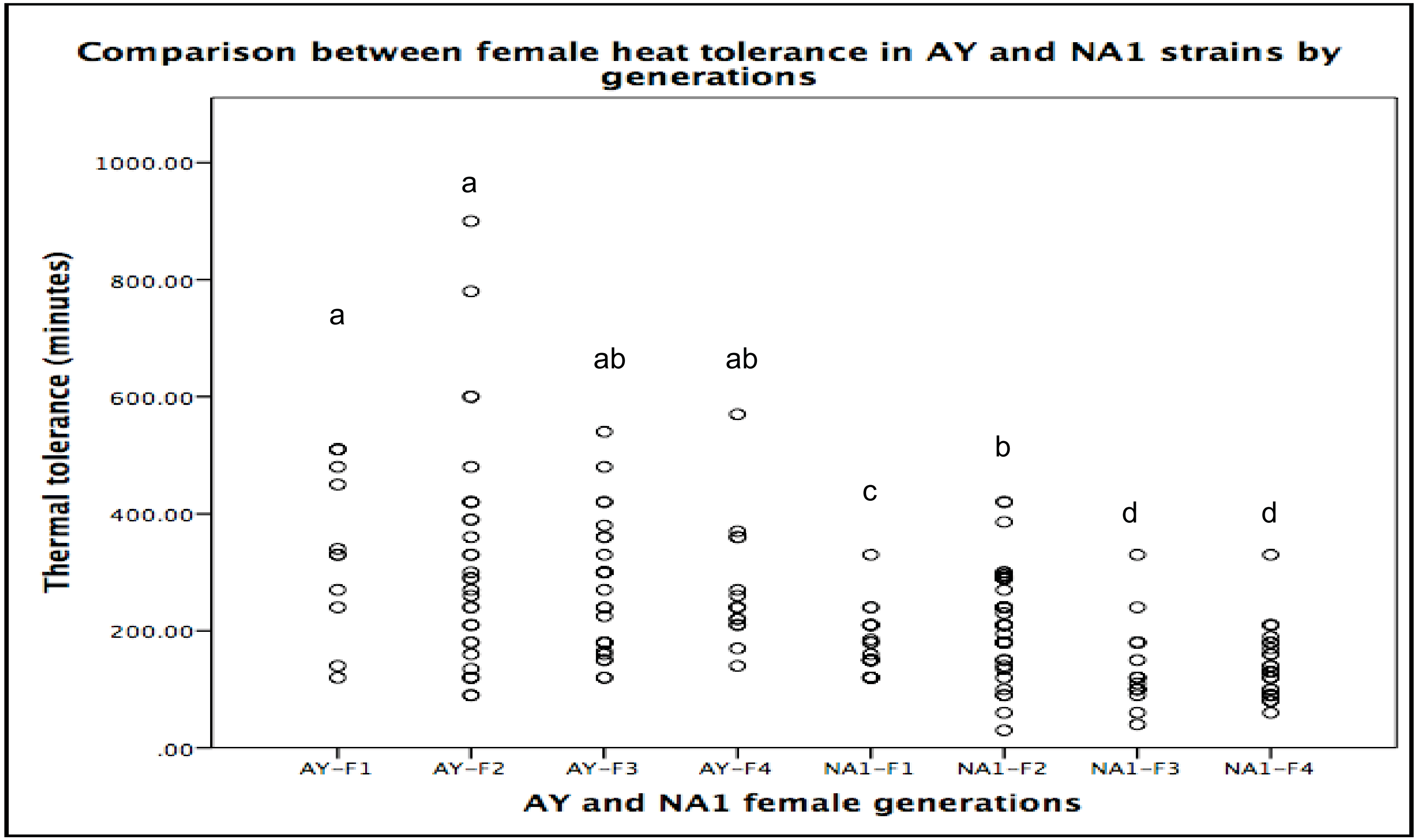

Figure 1.1- Boxplot of comparison between female thermal tolerance in AY and NA1 strains. Significant tolerances were observed between AY and NA1 compared by each generation. Decreases in thermal tolerances were observed as the inbreeding generation progress. Small alphabet(x) = significant by group

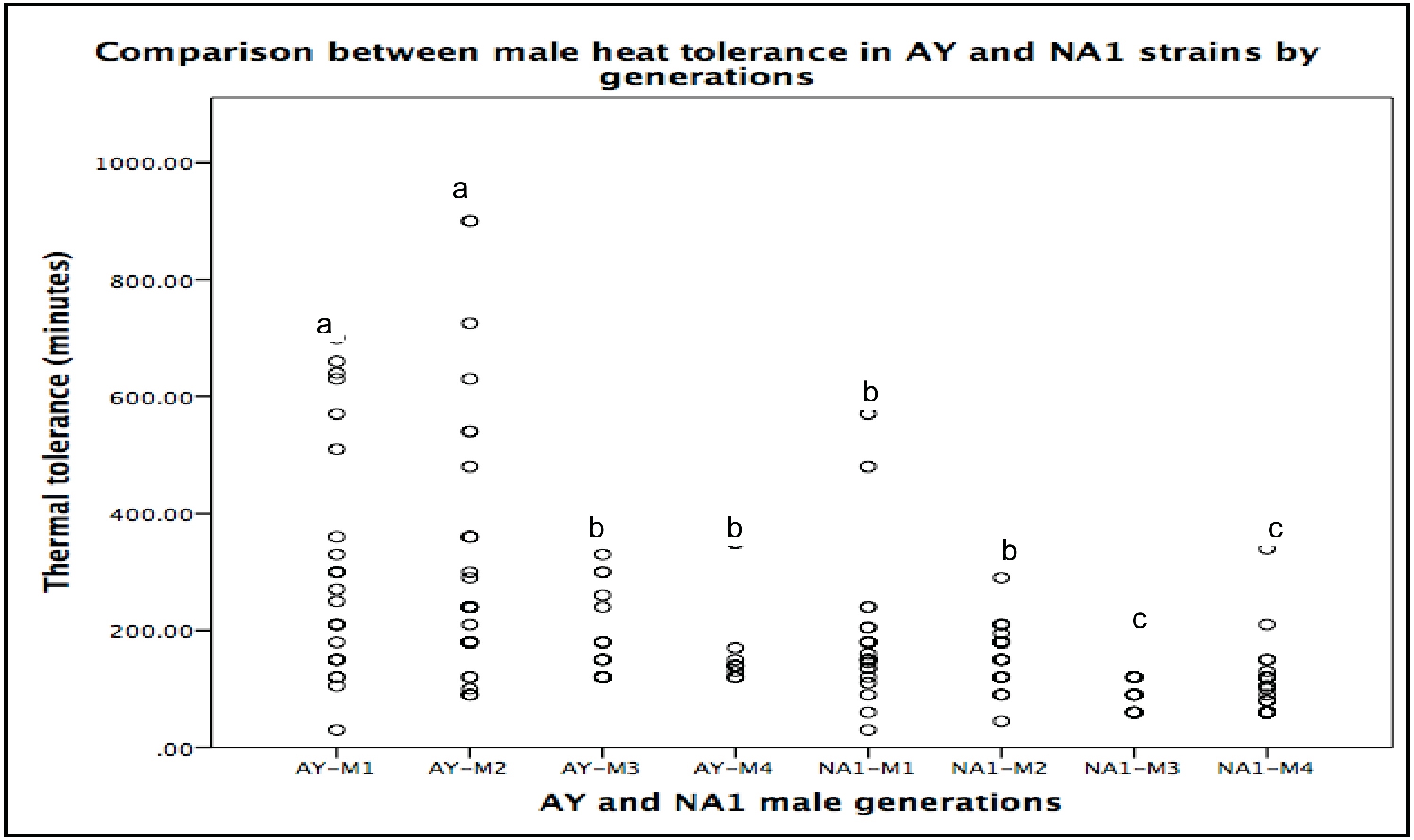

Figure 1.2- Boxplot of comparison between male thermal tolerance in AY and NA1 strains. AY strains have higher variations in thermal tolerance compared to NA1 male. Significant tolerances were observed between AY and NA1 compared by each generation. Small alphabet(x) = significant by group

Table 1.2 – Genetic changes in inbred AY strain guppy.

Strains	N	**Ho (SE)**	**He (SE)**	Fst	f	I
AY-1	**30**	0.522 (0.062)	0.626 (0.064)	0.102	0.250	1.287
AY-2	**60**	0.452 (0.127)	0.582 (0.057)	0.158	0.375	1.160
AY-3	**45**	0.382 (0.057)	0.508 (0.057)	0.233	0.500	0.998
AY-4	**26**	0.282 (0.088)	0.39 (0.074)	0.230	0.590	0.667
AY-3D	**13**	0.216 (0.061)	0.395 (0.028)	0.458	0.500	0.626
AY-4D	**15**	0.222 (0.073)	0.466 (0.032)	0.519	0.590	0.695
AY-1	**30**	0.522 (0.062)	0.626 (0.064)	0.102	0.250	1.287
AY-2	**60**	0.452 (0.127)	0.582 (0.057)	0.158	0.375	1.160
AY-3+D	**58**	0.355 (0.058)	0.494 (0.044)	0.266	0.500	0.957
AY-4+D	**41**	0.244 (0.070)	0.415 (0.035)	0.455	0.590	0.676

Notes: N= sampled individuals, Ho= observed Heterozygosity, He= expected heterozygosity, Fst= Fixation index, **I**= Shannon's information index. AY-x = AY strain x generations, AY-xD= dead AY strain at x generations. AY-x+D= Sum all live and dead samples at x generations. SE= standard error.

Table 1.3– Genetic changes in inbred NA1 strain guppy.

Strains	N	**Ho (SE)**	**He (SE)**	Fst	f	I
NA1-1	**45**	0.479 (0.055)	0.643 (0.062)	0.245	0.250	1.430
NA1-2	**75**	0.371 (0.055)	0.601 (0.072)	0.375	0.375	1.313
NA1-3	**34**	0.285 (0.075)	0.531 (0.065)	0.453	0.500	1.036
NA1-4	**49**	0.195 (0.043)	0.528 (0.089)	0.562	0.590	1.008
NA1-3D	**17**	0.21 (0.052)	0.522 (0.071)	0.545	0.500	0.948
NA1-4D	**26**	0.192 (0.059)	0.445 (0.077)	0.495	0.590	0.812
NA1-1	**45**	0.479 (0.055)	0.643 (0.062)	0.245	0.250	1.430
NA1-2	**75**	0.371 (0.055)	0.601 (0.072)	0.375	0.375	1.313
NA1-3+D	**51**	0.273 (0.071	0.578 (0.074)	0.526	0.500	1.145
NA1-4+D	**75**	0.186 (0.041)	0.489 (0.079)	0.556	0.590	0.936

Notes: N= sampled individuals, Ho= observed Heterozygosity, He= expected heterozygosity, Fst= Fixation index, **I**= Shannon's information index. NA1-x = NA1 strain x generations, NA1-xD= dead AY strain at x generations. NA1-x+D= Sum all live and dead samples at x generations. SE= standard error.

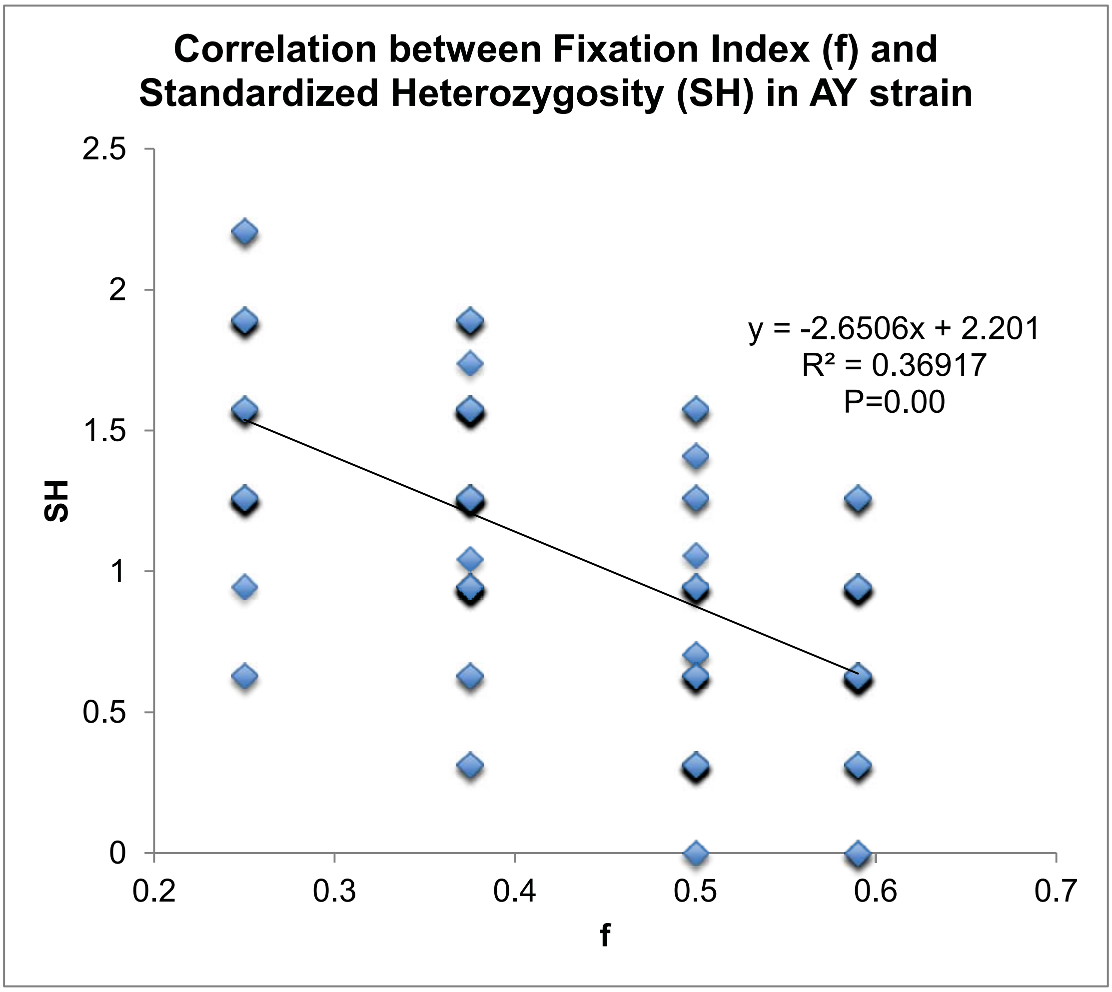

Figure 1.3 – The relationship between inbreeding coefficient (f, based on pedigree) and standardized heterozygosity in AY strain. Significant correlations were observed when comparison we made ($p<0.05$).

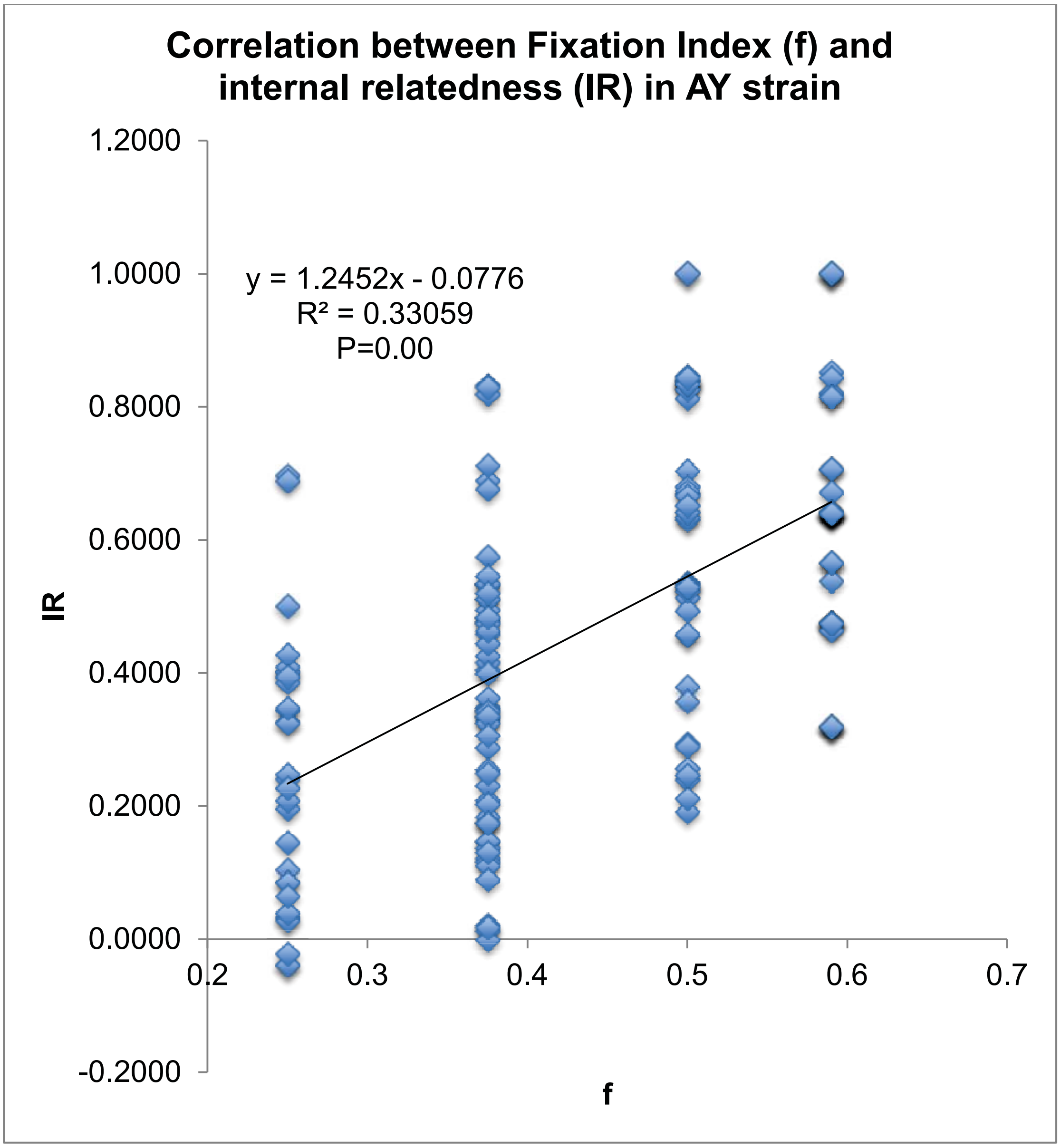

Figure 1.4 – The relationship between inbreeding coefficient (f, based on pedigree) and internal relatedness (IR) in AY strain. Significant correlations were observed when comparison we made ($p<0.05$).

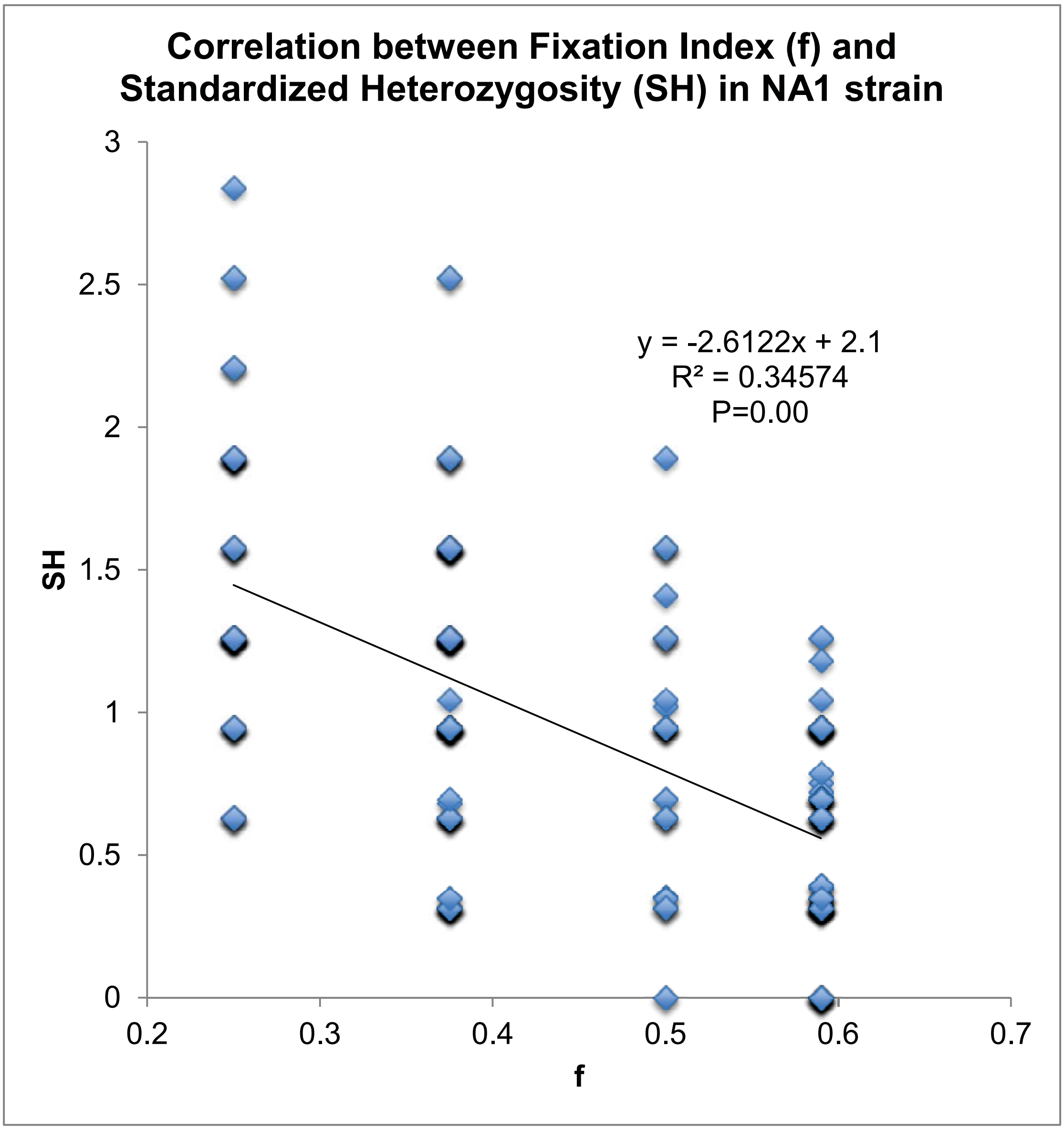

Figure 1.5 – The relationship between inbreeding coefficient (f, based on pedigree) and standardized heterozygosity in NA1 strain. Significant correlations were observed when comparison we made (p<0.05)

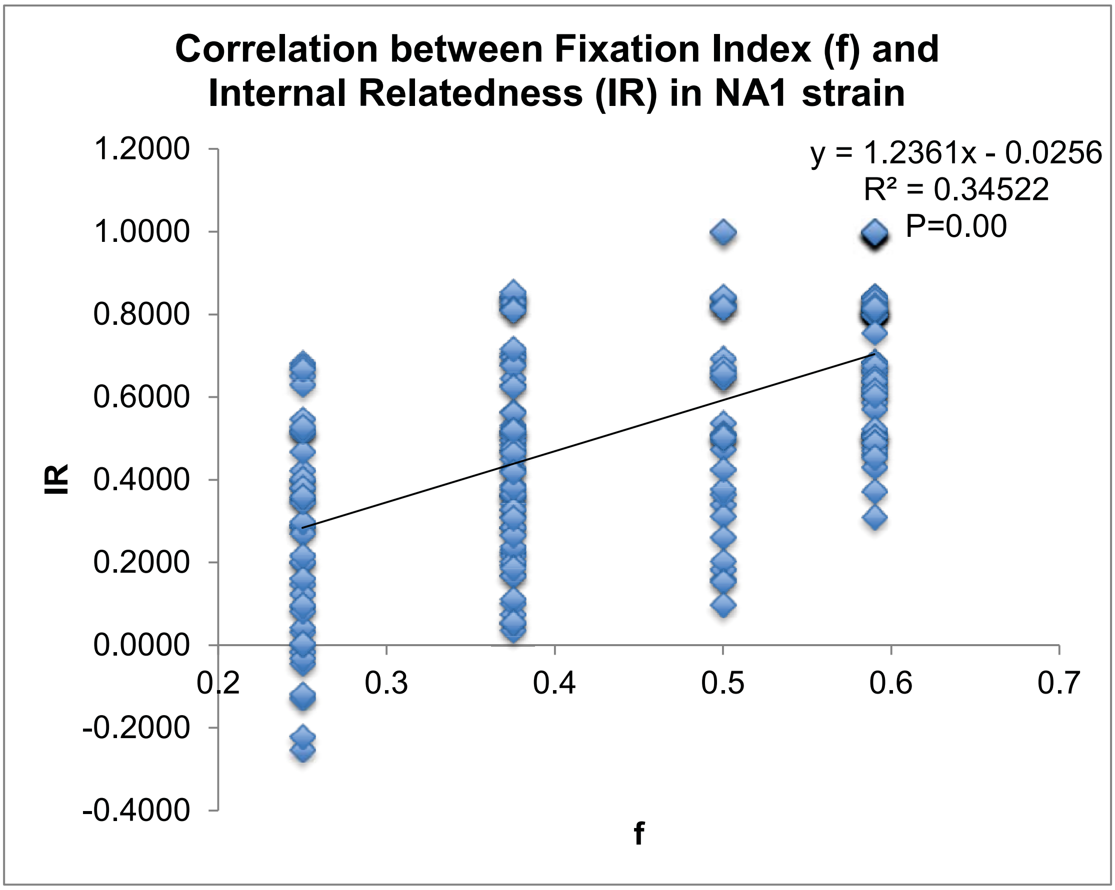

Figure 1.6 – The relationship between inbreeding coefficient (f, based on pedigree) and internal relatedness (IR) in NA1 strain. Significant correlations were observed when comparison we made ($p<0.05$).

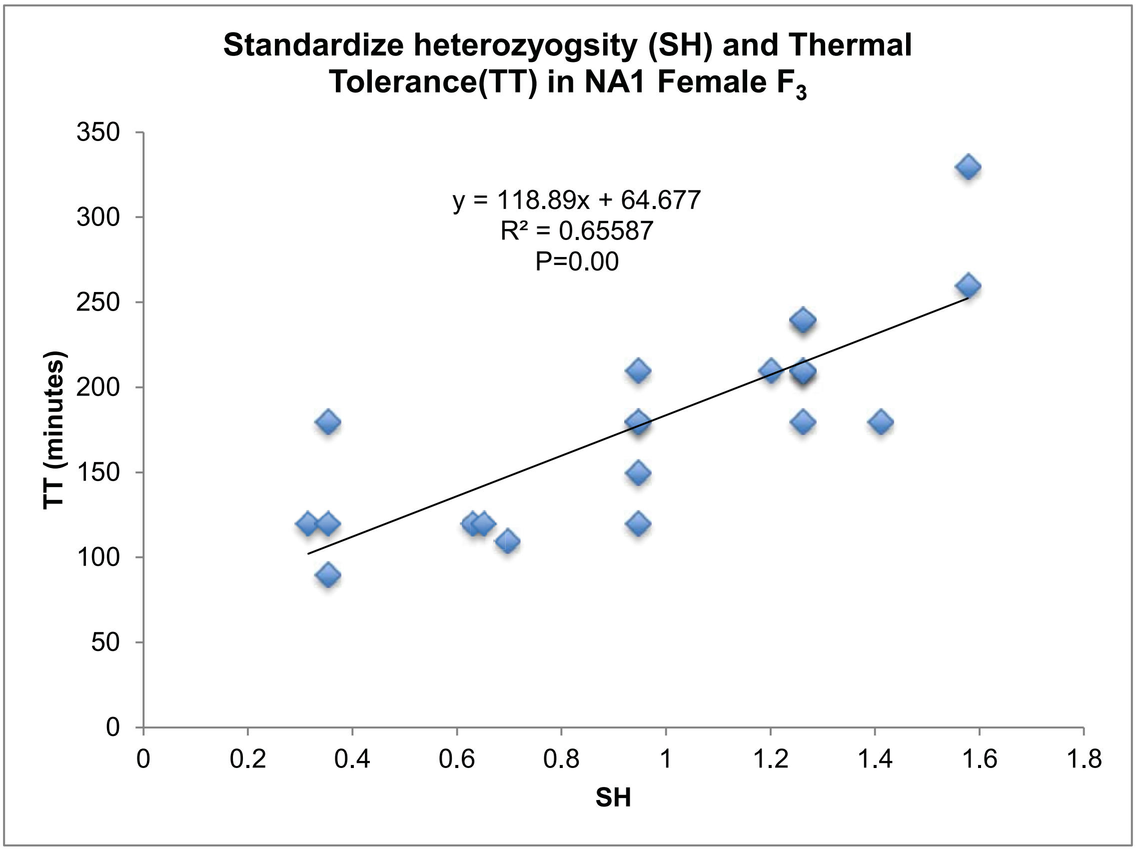

Figure 1.7 - Correlations between standardized heterozygosity (SH) with thermal tolerance in NA1 female F_3. Positive correlation between SH and TT. Third generation of female NA1 was significantly correlated ($p<0.05$).

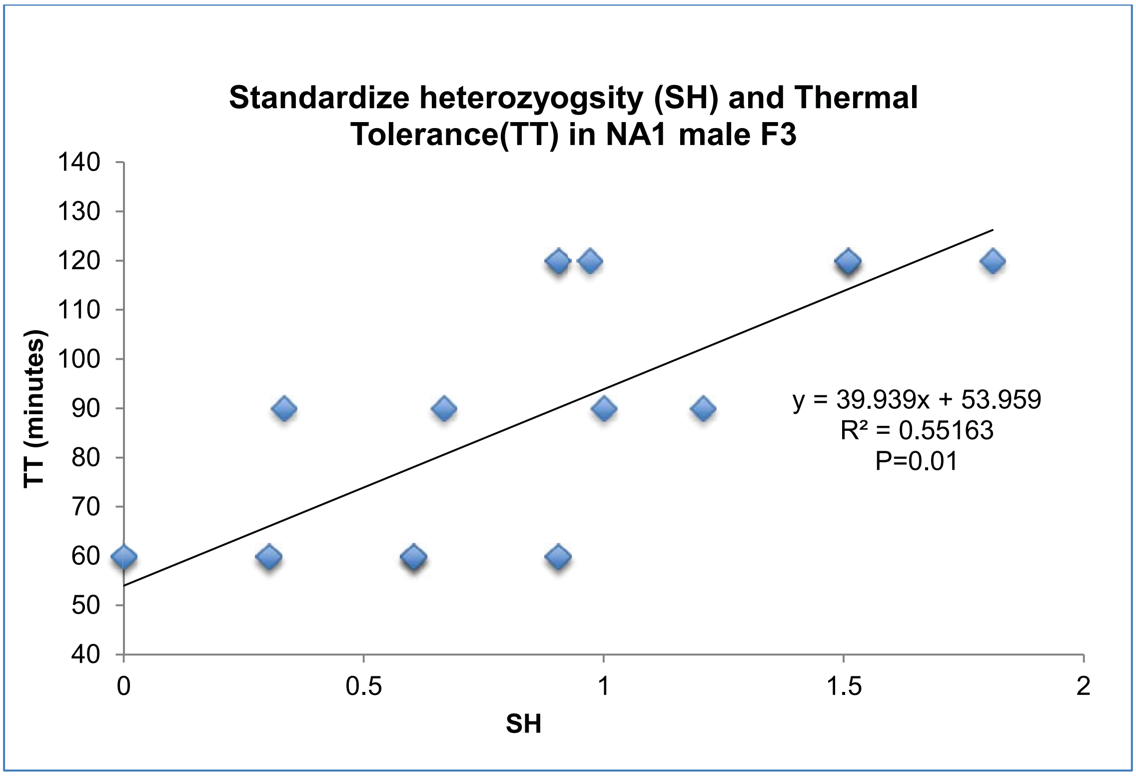

Figure 1.8 - Correlations between standardized heterozygosity (SH) with thermal tolerance in NA1 male F_3. Positive correlation between SH and TT were observed. Third generation of male NA1 was significantly correlated ($p<0.05$).

論文審査の結果の要旨及び担当者

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論　文　審　査　の　結　果　の　要　旨

近年、水産生物における生産量の約 50%は養殖により生産されている。栽培漁業などを含めると人為操作を経た生産量の割合はさらに高いものとなる。養殖や栽培漁業で問題となるのは少数親魚からの再生産による遺伝的多様性低下、近親交配の問題である。近親交配は遺伝的多様性の低下をもたらすとともに有害遺伝子の発現などの近交弱勢と呼ばれる様々な形質における劣化をもたらす。本研究はそのような近交弱勢を親魚の遺伝的多様性や遺伝的類縁関係から予測し予防する手法の開発を目的としている。

この研究ではグッピーをモデル実験魚として選び、兄妹交配を重ねることにより近交係数を上昇させ、前世代の親魚の遺伝的変異性や遺伝的類縁関係と次世代の環境適応能力、成長、再生産能力の比較を行っている。用いた系統は AY 系統と Na-1 系統である。いずれも野生化集団を起源としており観賞魚として育成された系統よりも高い遺伝的変異性を有している。遺伝マーカーにはマイクロサテライト DNA マーカーを用い、環境適応能力の指標として 180 日目生残率と高温耐性、再生産能力の指標として初産日数と初産時産仔数を用いている。

近交世代を重ねるにつれて遺伝的変異性は低下し、形質も体長を除き低下が観察された。親魚における遺伝的変異性と仔魚の高温耐性を比較したところ、Na-1 系統では有意な正の相関が観察され、親の変異性が高いほど子の高温耐性が高い関係を示した。しかし、この関係は AY 系統では観察されなかった。一方、両親の遺伝的類似度と仔魚の高温耐性との関係を調べたところ AY 系統で有意な負の相関が観察された。これは両親間の遺伝的類似度が低いほど仔魚の高温耐性が高いことを示している。一方、Na-1 系統でこのような関係は観察されなかった。Na-1 系統は AY 系統に比べ継代飼育期間が長く、AY 系統に比べ遺伝的変性は低い。そのため親個体間の遺伝子型の差異が反映されにくく、親の遺伝的類似度と高温耐性との間に有意な相関が観察されなかったものと考えられる。一方、AY 系統では様々な遺伝子型が存在し、ヘテロ接合体率だけで親世代の変異性を反映しきれなかったものと考えられた。以上のことから、集団の遺伝的変異性と親魚間の遺伝的類似度を用いることにより次世代での近交弱勢をある程度予測でき、予防が可能と結論している。

近交弱勢の予測と予防は増養殖において重要な課題であり、本研究で得られた知見はこれらの課題の解決に対して大きく貢献できると考えられる。以上のことから、審査委員一同は本研究に対し博士の学位を授与する価値があるとの判断で一致した。